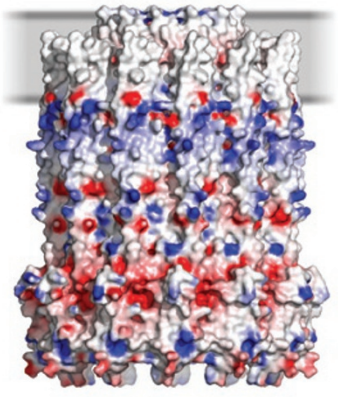
A new look at pore formation



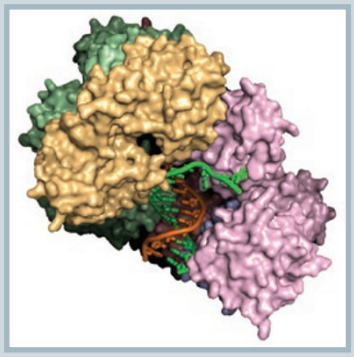
Bacterial pore-forming toxins (PFTs) are secreted virulence factors that assemble on host cell membranes and form water-filled transmembrane pores. These pores can disrupt chemical gradients and, at high concentrations, perturb membrane stability. They can also serve to transport effector molecules to the cell interior. PFTs are excellent model systems to study membrane protein folding because they begin as water-soluble monomers that spontaneously assemble to form oligomeric pores lined by α-helices (α-PFT) or a β-barrel (β-PFT) in target membranes. Of these two classes, a high-resolution structure of an assembled PFT pore is known only for α-hemolysin, a β-PFT from *Staphylococcus aureus*. Now, Ban and colleagues have determined the crystal structure of the dodecameric transmembrane pore formed by cytolysin A (ClyA), a cytotoxic α-PFT found in some *Escherichia coli* and *Salmonella enterica* strains. The pore is 130 Å high with an outer diameter of 105 Å at its widest point; the membrane-spanning region is ~30 Å high with inner and outer diameters of ~35 Å and ~90 Å, respectively. Each protomer has a three-helix bundle core that lines the pore, and the membrane-spanning region is composed primarily of helix αA. Comparison with the previously determined ClyA monomer structure reveals that each protomer undergoes major conformational changes upon pore formation, the most significant being the conversion from a four- to a three-helix bundle in the protomer core and a β-strand–to–α-helix change at the β-tongue, a region rich in hydrophobic residues. The authors suggest that the latter conversion is crucial for membrane association and for triggering the conformational change required for assembly and membrane insertion. In contrast to the β-PFTs, the structural analysis of the α-PFT indicates that most structural changes occur before the conversion from pre-pore to pore. (*Nature*, advance online publication, doi:10.1038/nature08026, 6 May 2009) *MM*

Location, location, location

Actin filament dynamics enable the cell to respond to a changing environment. *In vivo*, actin filament disassembly is promoted by cofilin, together with other proteins including coronin; *in vitro*, however, coronin has been found to inhibit actin filament disassembly. An explanation for these disparate observations was provided in a recent study by Gandhi *et al*. Coronin has an N-terminal actin-binding (β-propeller) domain and a C-terminal coiled coil (CC) domain. After confirming that coronin increases actin turnover mediated by cofilin *in vivo* but antagonizes cofilin-mediated filament severing *in vitro*, Ghandi and colleagues characterized the behavior of two truncation mutants: one contained the β-propeller and mid-domains but not the CC domain; the other contained just the CC domain. Surprisingly, the CC domain could inhibit cofilin on its own, suggesting that it also contained an actin-binding domain. In addition, the mutant lacking the CC domain facilitated cofilin-mediated filament severing *in vitro*, thus mimicking the *in vivo* behavior. This synergy did not occur through direct interaction between cofilin and the coronin N-terminal mutant but required the binding of actin through the mutant's β-propeller domain. These inconsistencies were resolved when the two actin-binding domains were characterized further. Actin filaments undergo unidirectional growth, so that the new leading end is an environment with ATP-bound or ADP+Pi-bound actin, whereas the trailing end contains ADP-actin. The authors found that the interaction of coronin with these two states of actin was different. The CC domain of full-length coronin binds the ATP end of the actin filament more tightly and blocks cofilin binding, thereby preventing filament severing at the growing end. At the other end, however, a weaker interaction of the coronin β-propeller domain with ADP-actin allows cofilin binding, such that its filament-severing activity is focused toward the trailing end. (*Mol. Cell* **34**, 364–374, 2009) *AKE*

Spiral binding

Sliding clamps are needed at primer-template junctions to attach the polymerase to DNA during chain extension. These sliding clamps are loaded onto the DNA by clamp loaders, which are ATPases.



The crystal structure of an ATP-loaded yeast replication factor C complex, which is related to bacterial clamp loaders, was previously solved bound to the proliferating cell nuclear antigen (PCNA) sliding clamp. The complex suggested a notched screw-cap model, in which ATP binding was proposed to induce a spiral arrangement of ATPases subunits that 'screws' onto the double-helical DNA; however, this model did not explain the full mechanism of the clamp loader. Now, Simonetta *et al*. provide the crystal structure of the *Escherichia coli* clamp loader complex bound to primer-template DNA and to a segment of the ψ protein. The ψ protein is not essential for clamp loader activity, but it enhances the binding of the loader to the sliding clamp and DNA and stimulates DNA-dependent ATP hydrolysis. This new structure reveals that the previously proposed notched screw-cap model is roughly correct. It also reveals a highly symmetric arrangement of the ATPase subunits and that each of the ATP binding sites is primed for catalysis. This supports the 'suicide complex' idea, in which ATP hydrolysis triggers conversion to an inactive conformation and breaks down the spiral. Surprisingly, the authors show that the contacts between the protein and the DNA are restricted to the template strand. This explains why clamp loaders can recognize both RNA and DNA primers. (*Cell* **137**, 659–671, 2009) *MH*

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